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(54) Title: MICRONUTRIENT PHOSPHATES AS DIETARY AND HEALTH SUPPLEMENTS

(57) Abstract: There is provided a dietary or health supplement comprising an effective amount of a micronutrient selected from the group consisting of phosphate derivatives of tocopherol, ubiquinol, ascorbic acid, tocotrienol, retinol and mixtures thereof delivered with an acceptable carrier.

MICRONUTRIENT PHOSPHATES AS DIETARY AND HEALTH SUPPLEMENTS

Field of the invention

The invention relates to dietary or health supplements for improved delivery of micronutrient compounds. More particularly, the invention relates to dietary or health supplements for improved delivery of micronutrient compounds which are electron transfer agents.

Background of the invention

In this specification, where a document, act or item of knowledge is referred to or discussed, this reference or discussion is not an admission that the document, act or item of knowledge or any combination thereof was at the priority date:

(a) part of common general knowledge; or

(b) known to be relevant to an attempt to solve any problem with which this specification is concerned.

Whilst the following discussion mainly concerns ubiquinol and tocopherol, it is to be understood that this is merely illustrative and that the invention is not limited to these electron transfer agents.

Coenzyme Q10 (CoQ_{10}) or ubiquinone is lipophilic because it has ten repeating isoprene units. It is an endogenous essential cellular constituent that is present in every cell of the body and serves as a coenzyme for several key steps in the production of energy within the cell. CoQ_{10} is regarded as being of fundamental importance as it is reported to play an important physiological role in the mitochondrial transport of electrons and production of energy within mitochondria of each cell.

CoQ_{10} is made available to the body through endogenous biosynthesis and dietary intake. Folkers suggests that a CoQ_{10} deficiency leading to evidence of a clinically significant disease state may occur because of:

- insufficient dietary CoQ_{10}
- impairment in CoQ_{10} biosynthesis,
- excessive utilization of CoQ_{10} by the body,

or any combination of the three. As CoQ_{10} is essential to the optimal function of all cell types, it is not surprising to find a seemingly diverse number of disease states which respond favorably to CoQ_{10} supplementation. All metabolically active tissues may therefore be highly sensitive to CoQ_{10} deficiency. It is reasonable to assume that optimal

nutrition (which may in future include optimal levels of CoQ_{10}) is generally beneficial in many disease states.

As CoQ_{10} improves the cell respiratory chain and stabilizes mitochondrial membranes, it has a potential role in some cardiac insufficiency diseases associated with aging. CoQ_{10} is known to be highly concentrated in heart muscle cells due to the high energy requirements of this cell type. Whether primary, secondary or both, this deficiency of CoQ_{10} may be a major treatable factor in the otherwise inexorable progression of heart failure. Other nutrients reported to be of potential use in treatment of cardiovascular diseases including vitamin E, inosine, cytochrome C, or treatment of hyperhomocysteinemia with oral folate, betaine and/or pyridoxine therapy could also be considered. These nutrients may assist in treating a range of diseases associated with age and stimulate endogenous CoQ_{10} production.

Dietary supplement

CoQ_{10} shows a high variability in its absorption, with some subjects attaining good blood levels of CoQ_{10} on 100 mg per day while others require two or three times this amount to attain the same blood level. All CoQ_{10} presently available in the United States is manufactured in Japan and is distributed by a number of companies who place the CoQ_{10} either in pressed tablets, powder-filled capsules, or oil-based soft gel capsules. CoQ_{10} is fat-soluble and absorption should be improved when administered with dietary fat. Published data on the dosage of CoQ_{10} relates almost exclusively to the treatment of disease states. There is no information on the use of CoQ_{10} for prevention of illness. This is an extremely important question which, to date, does not have an answer.

Absorption is reported to take place through:

(a) the formation of micelles with biliary salts in a similar fashion to vitamin A; or

(b) after direct adhesion of CoQ_{10} to the intestinal membrane, by passive transport (that is strongly inhibited by its high molecular weight); and/or

(c) lipoprotein transport.

However, when administered orally, absorption is highly variable and dependent upon formulation parameters.

CoQ_{10} is a poorly soluble quinone. Intestinal absorption of CoQ_{10} can be improved with effective formulation. There is evidence that fat soluble nutrients are better absorbed from aqueous or emulsified vehicles than from oily preparations.

Formulation	Daily Dose	Baseline CoQ10 Blood level	Peak CoQ10 Blood level	% Change (@ C_{Max})
Powder (particle size <125µm)	100 mg capsule	16525±1598 µg l^{-1}·h	21197±2046 µg l^{-1}·h	28%
Powder	60 mg tablet	0.45 µg/ml	0.98 µg/ml	118%
Powder	100 mg capsule	630±165 nmol/L	736±156 nmol/L	17%
Granular	90 mg	1.08±0.31 µmol/l	1.81±0.82 µmol/l change	168%
Oil suspension	120 mg soft gel	0.40±0.11 µg/ml	1.26±0.50 µg/ml	215%
Oil base	90 mg capsule	1.07±0.34 µmol/l	1.90±0.97 µmol/l change	178%
Oil base	90 mg soft gel	0.98±0.29 mg/l	2.03±0.58 mg/l	107%
Oil suspension (Glyceryl monoleate)	100 mg soft gel	16525±1598 µg l^{-1}·h	24941±3528 µg l^{-1}·h	51%
Lipid Microsphere (Soy oil+egg phospholipids)	60 mg soft gel	0.70 µg/ml	2.62 µg/ml	274%
Oil emulsion (MCT+surfactant)	100 mg soft gel	605±121 nmol/L	1534±384 nmol/L	253%
Oil emulsion (MCT+surfactant+Vit E)	120 mg soft gel	0.38±0.11 µg/ml	2.80±0.80 µg/ml	637%
Oil emulsion (Soy lecithin+Gelucire)	100 mg capsule	16525±1598 µg l^{-1}·h	52857±1948 µg l^{-1}·h	220%
Plasma Vit E & Vit C		-	-	No change

Despite these increases in bioavailability, it is important to note that absolute absorption still remains less than optimal. It is suggested that the increase in bioavailability noted in the above table is due to the oil emulsion surfactant system increasing solubility and dissolution rate, or the ability of surfactants to penetrate and disrupt biological membranes increasing permeability of the drug load. This assumes that uptake through the intestinal membrane is a passive process.

A leading American CoQ_{10} (Q-Gel) soft gelatine capsule uses a proprietary formula containing CoQ_{10} in a blend of sorbitan monooleate, polysorbate 80, medium chain triglycerides (MCT's), propylene glycol, d-alpha tocopherol, PVP (Plasdone) and annato seed extract which has been reported to increase CoQ_{10} bioavailability compared to other commercial formulations in US patent no 6,056,971.

Other methods of solubilising CoQ_{10} have been reported including hydrogenated castor oil (HCO-60) and ethanol-water (1:5 by vol) and other lipoidal drug delivery systems incorporating stable sub micron range particles in lecithin as discussed in US patent no 5,989,583. Lipid microspheres may enhance the absorption of CoQ_{10}.

Other parameters important to CoQ_{10} absorption include:

(a) awareness of the micelle size as a function of bioavailability,

(b) HLB value of surfactants as a function of bioavailability, as high HLB numbers may improve bioavailability (Pozzi et al. 1991, Weis et al. 1994), and

(c) CoQ10 granule size.

The intestinal absorption of lipid-soluble drugs can be markedly influenced by the oral dosage form as well as the formulation factors. Many commercial vitamin preparations are formulated as compressed tablets, hardshell gelatin capsules or soft gelatin capsules which contain a complex matrix of excipients, fillers and other adjuvants. Compounds formulated in soft gelatin capsules representing liquid fills tend to be better absorbed than hard gelatin capsules, which encapsulate a dry powder blend, however little attention has historically been given to bioavailability of dietary supplements.

In a recent study conducted by Walhqvist, the bioavailability of CoQ_{10} from two different preparations was compared in order to ascertain if the emulsified preparation had higher bioavailability of CoQ_{10} than a powdered preparation. Two different gelatin capsules containing 50 mg of CoQ_{10} were used in this study. The first preparation was crystalline CoQ_{10}, with dicalcium phosphate as a filler and magnesium stearate as an excipient, filled in a hard gelatin capsule. The other contained CoQ_{10} as a complex micelle in an emulsion encapsulated into a soft gelatin capsule. The conclusion of the study was that the emulsified soft gel capsule had a higher bioavailability than the powder in hard gel formulation used in this study. The presence of surfactants in the soft gel formulation would contribute to the enhanced solubilisation and release of CoQ_{10}.

Despite all this research into delivery of CoQ_{10}, the absorption levels which have been achieved are not yet optimal.

Foods with additional micronutrients

Appreciation of dietary CoQ_{10} intake is important when considering formulation of dietary supplements and functional foods for a number of reasons. The chemistry of the compound is also important and can indicate preferred forms utilized by the body. Vitamin B6 for example, can be found in the free form (pyridoxine), a glycoside (pyridoxamine) and supplied in dietary supplements as a hydrochloride salt (pyridoxine hydrochloride). In foods, the vitamin primarily exists as a phosphate (pyridoxal 5-phosphate). Bioavailability varies depending upon the type of food and method of preparation but typically the phosphate is better ingested.

Finished product formulations should be representative of the original food source to be certain that other compounds accompanying CoQ_{10} originally present in the food are

present. These dietary compounds are important to consider as they can dramatically alter bioavailability of the finished product. So where possible consideration should be given to what compounds are also present. For example foods rich in CoQ_{10} are typically fatty eg: oily fish and soy oil. It is therefore not surprising then that bioavailability of CoQ_{10} is reported to improve when formulated with a lipid vehicle.

Consideration of food sources rich in CoQ_{10} will help identify normal dietary intake levels. In peer reviewed literature there is some variance in opinion on what constitutes an adequate or effective dose of CoQ_{10} and some thought that dietary intake could uniformly be low.

Food preparation is also important to consider and can assist with knowing how these methods affect absorption. For example, the effect of cooking is a 14-32% destruction of CoQ_{10} by frying, and no detectable destruction by boiling. This suggests that CoQ_{10} is likely to be heat stable, may be utilized in hot beverages and is likely to be successfully concentrated by moderate heat extraction.

Regular food intakes are important to consider and indicate that in normal individuals a low intake is adequate or that bioavailability is optimal because naturally co-administered compounds present in the food improve absorption.

Reduced CoQ_{10} (ubiquinol) delivered in supplements has been reported to increase circulating levels of reduced CoQ_{10}. How the molecule is changed into an oxidised form is becoming clearer. Analysis of the actual form of ubiquinol in foods has not been undertaken, nor is it clear what form is preferred by the body. However, it is thought that to act effectively as an electron transfer agent CoQ_{10} must remain in a reduced form.

There is thus a need for an improved delivery system for micronutrient compounds such as reduced CoQ_{10} and other important dietary or health supplements.

Vitamin E

Vitamin E is a potent electron transfer agent capable of protecting polyunsaturated fatty acids (PUFA) within phospholipids of biological membranes and plasma lipoproteins. Vitamin E also stabilizes membranes, modulates protein kinase C activity and positively influences immune response. Although supplementation is popular, only higher dietary consumption is reliably associated with lower risk of coronary heart disease in both men and women on a cross cultural basis.

When provided as a supplement, vitamin E is provided as tocopherol. When delivered as an isolated nutrient, vitamin E is poorly absorbed due to its lipid solubility and chemically unstable due to primary oxidation of the phenolic group. To improve delivery, vitamin E is

esterified and presented as simple substituted esters - either succinate or acetate derivatives. While this pro-drug strategy is primarily undertaken to prevent oxidation of the phenolic group, improve lymphatic transport, and enhance stability, increase in tissue tocopherol may take many weeks to achieve.

Although dietary supplementation with vitamin E esters - particularly the natural form - *RRR*- stereoisomer, may increase the content of α-tocopherol in blood plasma and erythrocytes, bioavailability is still significantly less than optimal with blood levels being subject to wide inter-patient variability and clinical efficacy disappointing.

Luminal events in gastrointestinal lipid digestion have been well studied and a micellar hypothesis of fat absorption established. A number of attempts have therefore been made to enhance α-tocopherol acetate lymphatic transport via lipid formulation approaches. Despite improvements, food can still have a significant impact increasing the extent of α-tocopheryl ester absorption after oral administration, indicating that factors other than dispersion, digestion and solubilisation may be responsible for intestinal uptake of vitamin E. Other lipophilic drugs and nutrients are also subject to poor and variable absorption properties following oral administration including vitamin A, indicating that current self emulsifying drug delivery formulation approaches as well as other lipid-based formulations may be of limited value in increasing bioavialability of poorly soluble lipid compounds.

Being fundamentally important to cellular viability, vitamin E must be transported efficiently and mobilised on demand to act as an electron transfer agent and not reach too high a concentration to become pro-oxidant. This delicate biological balance must start with effective transport across the small intestine mucosa, yet this process is currently not well understood.

Tocopheryl phosphate (TP) is a more water-soluble analogue of tocopherol and proposed to have higher bioavailability than tocopheryl acetate (TA) most likely because of more efficient intestinal uptake. As a water-soluble analogue TP is easier to formulate in functional foods, and dietary supplements but many enzymes in the gastrointestinal tract have phosphorylase activity and reduce the amount of TP delivered to the small intestine. TP also forms acid insoluble complexes that may reduce the amount of product available for transport across the intestinal wall.

There is a need for a delivery system which effectively provides improved delivery of a portion of the daily allowance of micronutrient compounds such as vitamin E and CoQ_{10}.

Summary of the invention

It has been discovered that the provision of a dietary or health supplement comprising micronutrient compounds is markedly improved by use of the micronutrient in the form of phosphate derivatives.

According to a first aspect of the invention, there is provided a dietary or health supplement comprising an effective amount of a micronutrient selected from the group consisting of phosphate derivatives of ubiquinol, ascorbic acid, tocotrienol, retinol and mixtures thereof delivered with an acceptable carrier.

Preferably, the micronutrient is ubiquinyl phosphate.

The term "phosphate derivatives" comprises compounds covalently bound by means of an oxygen to the phosphorus atom of a phosphate group. The oxygen atom is typically derived from a hydroxyl group on the micronutrient. The phosphate derivative may exist in the form of a free phosphate acid, a salt thereof, a phosphate ester having two molecules of micronutrient, a mixed phosphate ester having two different micronutrients, a phosphatidyl compound wherein the free phosphate oxygen forms a bond with an alkyl or substituted alkyl group and complexes with amphoteric surfactants, cationic surfactants, amino acids having nitrogen functional groups and proteins rich in these amino acids.

Preferably, the phosphate mixtures consist of one mono-micronutrient phosphate derivative and one di-micronutrient phosphate derivative wherein the amount of mono-micronutrient phosphate derivative is no less than equimolar to the amount of di-micronutrient phosphate derivative as disclosed in international patent application no PCT/AU01/01475. For example, a mixture containing 70% ubiquinyl phosphate and 26% di-ubiquinyl phosphate.

Phosphorylation may be accomplished by any suitable method. Preferably, the hydroxyl group-containing micronutrient is phosphorylated using P_4O_{10} according to the method in international patent application no PCT/AU00/00452. Excess diphosphate derivatives may be hydrolyzed using methods known to those skilled in the art.

In some situations, it may be necessary to use a phosphate derivative such as a phosphatide where additional properties such as increased water solubility are preferred. Phosphatidyl derivatives are amino alkyl derivatives of organic phosphates. These derivatives may be prepared from amines having a structure of $R_1R_2N(CH_2)_nOH$ wherein n is an integer between 1 and 6 and R_1 and R_2 may be either H or short alkyl chains with 3 or less carbons. R_1 and R_2 may be the same or different. The phosphatidyl derivatives are prepared by displacing the hydroxyl proton of the micronutrient with a phosphate entity

that is then reacted with an amine, such as ethanolamine or N,N' dimethylethanolamine, to generate the phosphatidyl derivative of the micronutrient. One method of preparation of the phosphatidyl derivatives uses a basic solvent such as pyridine or triethylamine with phosphorous oxychloride to prepare the intermediate which is then reacted with the hydroxy group of the amine to produce the corresponding phosphatidyl derivative, such as P cholyl P ubiquinyl dihydrogen phosphate.

According to a second aspect of the invention, there is provided a method for supplementing a subject's intake of a daily allowance of a micronutrient selected from the group consisting of ubiquinol, ascorbic acid, tocotrienol, retinol and mixtures thereof, said method comprising administering to said subject a dietary or health supplement comprising an effective amount of the micronutrient in the form of a phosphate derivative of the micronutrient delivered with an acceptable carrier.

Use of an effective amount of one or more phosphate derivatives of one or more micronutrients together with an acceptable carrier in the manufacture of a dietary or health supplement for supplementing a subject's intake of a daily allowance of the micronutrient, wherein the micronutrient is selected from the group consisting of ubiquinol, ascorbic acid, tocotrienol, retinol and mixtures thereof.

A dietary or health supplement when used for supplementing a subject's intake of a micronutrient selected from the group consisting of ubiquinol, ascorbic acid, tocotrienol, retinol and mixtures thereof, the dietary or health supplement comprising an effective amount of one or more phosphate derivatives of one or more micronutrients and an acceptable carrier.

According to a third aspect of the invention there is provided a dietary or health supplement comprising an effective amount of a micronutrient selected from the group consisting of complexes of phosphate derivatives of ubiquinol, ascorbic acid, retinol, tocotrienol, tocopherol and mixtures thereof delivered with an acceptable carrier.

The term "complexes of phosphate derivatives of a micronutrient" refers to the reaction product of one or more phosphate derivatives of ubiquinol, ascorbic acid, tocotrienol, retinol, tocopherol and mixtures thereof and one or more complexing agents selected from the group consisting of amphoteric surfactants, cationic surfactants, amino acids having nitrogen functional groups and proteins rich in these amino acids as disclosed in international patent application no PCT/AU01/01476.

The preferred complexing agents are selected from the group consisting of arginine, lysine and tertiary substituted amines, such as those according to the following formula:

$$NR^1R^2R^3$$

wherein R^1 is chosen from the group comprising straight or branched chain mixed alkyl radicals from C6 to C22 and carbonyl derivatives thereof;

R^2 and R^3 are chosen independently from the group comprising H, CH_2COOX, $CH_2CHOHCH_2SO_3X$, $CH_2CHOHCH_2OPO_3X$; CH_2CH_2COOX, CH_2COOX, $CH_2CH_2CHOHCH_2SO_3X$ or $CH_2CH_2CHOHCH_2OPO_3X$ and X is H, Na, K or alkanolamine provided R^2 and R^3 are not both H; and

wherein when R^1 is RCO then R^2 may be CH_3 and R^3 may be $(CH_2CH_2)N(C_2H_4OH)$-H_2CHOPO_3 or R^2 and R^3 together may be $N(CH_2)_2N(C_2H_4OH)CH_2COO$-.

Examples of such complexes of phosphate derivatives of a micronutrient are formed by the reaction of any combination of A) tocopheryl phosphate, retinyl phosphate, ascorbyl phosphate, tocotrienyl phosphate, ubiquinyl phosphate or mixtures thereof with B) arginine, lysine or lauryliminodipropionic acid where complexation occurs between the alkaline nitrogen center and the phosphoric acid ester to form a stable complex.

According to a fourth aspect of the invention, there is provided a method for supplementing a subject's intake of a daily allowance of a micronutrient selected from the group consisting of tocopherol, ubiquinol, ascorbic acid, tocotrienol, retinol and mixtures thereof, said method comprising administering to said subject a dietary or health supplement comprising an effective amount of the micronutrient in the form of one or more complexes of phosphate derivatives of the micronutrient delivered with an acceptable carrier.

The term "effective amount" refers to a portion or multiple of the daily allowance of each micronutrient which provides a bioactive effect on the subject. It is recognized that lipophilic substances are not readily excreted or metabolised so it is unusual to supply a large multiple of the recommended daily allowance (***RDA***) in a food source. It is recommended that typically any non medical use of dietary supplements should contain less than the recommended than the RDA and typically a third of the RDA. But it is recognised that for chronic medical uses, it is desirable to supply large multiples of the RDA for rapid increase in recovery.

The effective amount of the one or more phosphate derivatives of a micronutrient may be a concentration in the range of from 10 ppm to 10,000 ppm (w/w) of the dietary or health

supplement. Preferably, the one or more phosphate derivatives of a micronutrient is added at a concentration of 50 ppm to 1,000 ppm (w/w) in accordance with the need to supply the recommended daily allowance or a small multiple thereof.

The term "dietary or health supplement" as used in this description refers to all forms of supplying micronutrient compounds. For example, tablets, powders, chewable tablets, capsules, nasal delivery solutions, food additives, oral suspensions, children's formulations, enteral feeds, parenteral nutrition (for example, intravenous feeds), nutraceuticals and functional foods. Preferably, the dietary or health supplement is in a form selected from but not limited to the group consisting of capsule, tablet, powder and foods such as cookie, biscuit, breakfast cereal, sports drink and sports food bar. A person skilled in the art would know the acceptable carriers and other excipients which could be used in the invention. Typically, if the one or more phosphate derivatives of a micronutrient, such as ubiquinyl phosphate, is lipophilic then there is a lipidic carrier used such as medium chain triglycerides.

Use of an effective amount of a micronutrient together with an acceptable carrier in the manufacture of a dietary or health supplement for supplementing a subject's intake of a daily allowance of the micronutrient, wherein the micronutrient is selected from the group consisting of complexes of phosphate derivatives of ubiquinol, ascorbic acid, retinol, tocotrienol, tocopherol and mixtures thereof.

A dietary or health supplement when used for supplementing a subject's intake of a micronutrient, the dietary or health supplement comprising an effective amount of one or more complexes of phosphate derivatives of ubiquinol, ascorbic acid, retinol, tocotrienol, tocopherol and mixtures thereof and an acceptable carrier.

Examples

The invention will now be further explained and illustrated by reference to the accompanying non-limiting examples.

Example 1

In this example, ubiquinyl phosphate was prepared in a form suitable for use in supplements according to the invention.

100g ubiquinol was heated to 100 °C and 33g of P_4O_{10} was added. The mixture was stirred for 3 hours and 500 ml water was then introduced slowly into the mixture. The temperature of the reaction was maintained just below boiling point for a further 1 hour. Removal of water yielded ubiquinyl phosphate, and inorganic phosphates. The inorganic

phosphates were removed by further washes with hot water. The remaining amorphous material was then mixed with 100 L of virgin grade canola oil containing at least 1 to 5% lecithin. The final mixture of ubiquinyl phosphate in canola oil at a concentration of 1 mg/ml was incorporated into supplements such as capsules and functional foods.

Example 2

In this example, a capsule for use in increasing CoQ_{10} levels was prepared containing ubiquinyl phosphate according to the invention.

A suitably sized gelatin capsule (10 to 17 minum soft gel capsule or suitably sized dose form with 100 to 1000 mg fill) was selected from commercially available sources. One litre of the ubiquinyl phosphate lipidic mixture formed in Example 1 was then heated to 30°C prior to dispensing into a sealed soft gelatin capsule using known standard methods of soft gelatin capsule manufacture.

Example 3

In this example, a functional food for delivery of CoQ10 was prepared containing ubiquinyl phosphate according to the invention. In this case, ubiquinyl phosphate was incorporated into chocolate chip cookies.

One cup of butter or margarine incorporating 3 g ubiquinyl phosphate was creamed with 1 cup of brown sugar and 1 cup of plain sugar. One egg and 0.5 teaspoon of vanilla essence were then blended into the mixture. Two cups of plain flour was combined with 1.5 cups of oats, 1 teaspoon of baking powder and 300 grams of chocolate chips. Then the wet mixture was added to dry ingredients and mixed until a doughy consistency was obtained. Small balls were placed onto a greased tray allowing room to spread. The cookies were baked in a preheated oven at 180°C for 8 to 10 minutes. Approximately 30 biscuits were made with this recipe achieving 100 mg of ubiquinyl phosphate per serve. Variation of this amount may be considered to achieve the desired dosage.

Example 4

The preparation method for tocopheryl phosphate arginine complex is as follows:

The molar ratio of the compounds arginine, NaOH and mixture of tocopheryl phosphate/ditocopheryl phosphate (free acid form) is nominally 1:1:1, but a slight excess of arginine and NaOH was employed. A saturated solution of NaOH (60% w/w) was added to the dry arginine and stirred at 70°C for 20 minutes. Water (30 ml for every 150 g of TP/T_2P to be used) was added to facilitate better mixing. The tocopheryl

phosphate/ditocopheryl phosphate mixture was added to the solution and stirred vigorously with a high-shear mixer at 70°C for 1 hour.

Example 5

In this example, the stability of the tocopheryl phosphate arginine complex (***TP***) in a beverage was investigated.

Tocopheryl phosphate arginine complex (equivalent α-tocopherol content of 50 mg/500ml) was added to commercially available Musashi drinks (Musashi, Australia) a blue variety and an orange variety.

A 2% (w/v) tocopheryl phosphate arginine complex stock solution was prepared in water and filter-sterilised using a Millipore Millex-GP 25 mm, diameter 0.22 μm filter and sterile hypodermic syringe. Four ml was added to each 500 ml bottle using sterile conditions. Three treatment groups were used for each drink: 4°C, 37°C and room temperature (RT). The drinks were monitored for bacterial growth and contamination on a weekly basis, both visually, and by plating a sample onto LB agar plates, which were grown for 48 hours at 37°C.

The results showed that there was no bacterial or fungal contamination in any of the samples within each treatment group.

pH results:

Treatment group	**4°C**	**37°C**	**RT**
Blue group (no TP)	4.27	4.28	4.11
Blue group (TP)	4.34	4.31	4.16
Orange group (no TP)	3.40	3.27	3.26
Orange group (TP)	3.42	3.29	3.28

Turbidity Measurements

NTU results	**4°C**	**37°C**	**RT**
Blue group (no TP)	0.08	0.23	0.06
Blue group (TP)	12.54	28.55	24.05
Orange group (no TP)	218.0	190.0	206.0
Orange group (TP)	232.0	220.0	224.0

Drinks are made acidic to ensure microbial stability. There is detectable turbidation at low pH. However, at the pH required for biological stability, the amount of degradation of the tocopheryl phosphate arginine complex was negligible. This form of vitamin E supplementation is useful for such drinks.

Example 6

In this example, the bioavailability of tocopheryl phosphate in rats was investigated.

Method

The rats were dosed using the following protocol:

(a) A single dose of the compound was administered to male Sprague-Dawley rats (see Table 1). Oral gavage was with an 18 g gavage needle and 1 ml syringe. Intravenous was with a 26 g hypodermic needle and 1 ml syringe.

(b) Twenty four hours after administrations, the rats were anaesthetized with 60 mg/kg of Nembutal (an anaesthetic from Meril, USA) by intra peritoneal injection.

(c) Once the rats were under deep anaesthesia, a sample of blood was taken from the tail vein, and the femoral vein was exposed and injected with 500 units of heparin. The abdominal cavity was opened and the rat perfused with saline. The liver, heart, epidydimal fat pad, hind-leg muscle and brain was removed and frozen in liquid nitrogen.

The livers were extracted according to the following method:

(a) Homogenise 1 g of liver in 10 ml dichloromethane.

(b) Add 0.1 mg of ditocopheryl phosphate (1 mg/ml in 50% tetrahydrofuran) as an internal standard.

(c) Mix using homogeniser and centrifuge sample.

(d) Remove and evaporate dichloromethane.

(e) Add 9 ml of KOH (2M) and stir for one hour at room temperature.

(f) Add 10 ml hexane, shake and remove hexane (upper) layer.

(g) Add 10 ml HCl (2M) to the 9 ml KOH (2M) solution.

(h) Add 10 ml hexane, shake and remove hexane layer.

(i) Evaporate hexane layer to dryness.

The extracts were analysed and quantitated for TP (μg) content by electrospray mass spectrometry using the established calibration curve (tocopheryl phosphate vs ditocopheryl phosphate).

ES/MS analysis conditions: Sample was dissolved in 1 ml tetrahydrofuran (*THF*) containing 1% ammonia, 20 μl was injected into the sample loop. The sample was eluted with THF:water (9:1) 20 μl/min. Mass spectrometric analysis was conducted in negative ion mode with cone voltage of 40V using Micromass Platform.

Results

Table 1

Compound	Route	Dose (mg/kg)	Tocopheryl phosphate in liver (μg/g)
Tocopheryl phosphate	intravenous	10	24.0
Tocopheryl phosphate	intravenous	30	28.0
Tocopheryl phosphate	Oral (enteric coated)	30	19.3
Tocopheryl phosphate	oral	10	19.2
Tocopheryl phosphate	oral	30	18.4
Tocopheryl acetate	oral	10	12.8
Tocopheryl acetate	oral	30	13.7
Tocopheryl acetate	oral	100	14.0
Control (0.3 ml water)	intravenous	0	12.0
Control (0.3 ml corn oil)	oral	0	8.5

n=3 in all cases.

Conclusions

Rats were dosed with tocopheryl phosphate or tocopheryl acetate by oral and intravenous administration. Intravenous administration of tocopheryl phosphate led to a significant increase in the amount of tocopheryl phosphate present in the liver after 24 hours. The administration of tocopheryl phosphate as an enteric coating preparation of as an oral solution also led to an increase in the amount of tocopheryl phosphate detected in the

livers. The administration of tocopheryl acetate did not result in a significant increase in the amount of tocopheryl phosphate present compared to the controls.

The word 'comprising' and forms of the word 'comprising' as used in this description does not limit the invention claimed to exclude any variants or additions.

Modifications and improvements to the invention will be readily apparent to those skilled in the art. Such modifications and improvements are intended to be within the scope of this invention.

WHAT IS CLAIMED IS:

1. A dietary or health supplement comprising an effective amount of a micronutrient selected from the group consisting of phosphate derivatives of ubiquinol, ascorbic acid, tocotrienol, retinol and mixtures thereof delivered with an acceptable carrier.

2. A dietary or health supplement according to claim 1 wherein the micronutrient is ubiquinyl phosphate.

3. A dietary or health supplement according to claim 1 wherein the micronutrient is a phosphatidyl derivative of ubiquinol, ascorbic acid, tocotrienol, retinol and mixtures thereof.

4. A dietary or health supplement according to claim 1 wherein the one or more phosphate derivatives of a micronutrient is added at a concentration of from 10 ppm to 10,000 ppm % (w/w).

5. A dietary or health supplement according to claim 4 wherein the micronutrient is added at a concentration of from 50 ppm to 1,000 ppm % (w/w).

6. A dietary or health supplement according to claim 1 selected from the group consisting of capsule, tablet, powder, food additives, cookie, biscuit, breakfast cereal, sports drink and sports food bar.

7. A dietary or health supplement according to claim 6 being a capsule.

8. A dietary or health supplement according to claim 7 containing ubiquinyl phosphate.

9. A dietary or health supplement according to claim 6 being a sports drink.

10. A dietary or health supplement according to claim 9 containing ubiquinyl phosphate.

11. A dietary or health supplement according to claim 6 being a cookie containing ubiquinyl phosphate.

12. A dietary or health supplement according to claim 11 containing ubiquinyl phosphate.

13. A method for supplementing a subject's intake of a daily allowance of a micronutrient selected from the group consisting of ubiquinol, ascorbic acid, tocotrienol, retinol and mixtures thereof, said method comprising administering to said subject a dietary or health supplement comprising an effective amount of the

micronutrient in the form of a phosphate derivative of the micronutrient delivered with an acceptable carrier.

14. Use of an effective amount of one or more phosphate derivatives of one or more micronutrients together with an acceptable carrier in the manufacture of a dietary or health supplement for supplementing a subject's intake of a daily allowance of the micronutrient, wherein the micronutrient is selected from the group consisting of ubiquinol, ascorbic acid, tocotrienol, retinol and mixtures thereof.

15. A dietary or health supplement when used for supplementing a subject's intake of a micronutrient selected from the group consisting of ubiquinol, ascorbic acid, tocotrienol, retinol and mixtures thereof, the dietary or health supplement comprising an effective amount of one or more phosphate derivatives of one or more micronutrients and an acceptable carrier.

16. A dietary or health supplement comprising an effective amount of a micronutrient selected from the group consisting of complexes of phosphate derivatives of ubiquinol, ascorbic acid, retinol, tocotrienol, tocopherol and mixtures thereof delivered with an acceptable carrier.

17. A dietary or health supplement according to claim 16 wherein the micronutrient is selected from the group consisting of the reaction product of

(a) a micronutrient selected from the group consisting of tocopheryl phosphate, retinyl phosphate, ascorbyl phosphate, tocotrienyl phosphate, ubiquinyl phosphate or mixtures thereof; and

(b) a complexing agent selected from the group consisting of arginine, lysine or lauryliminodipropionic acid.

18. A dietary or health supplement according to claim 17 wherein the micronutrient is ubiquinyl phosphate arginine complex.

19. A dietary or health supplement according to claim 17 wherein the micronutrient is tocopheryl phosphate arginine complex.

20. A dietary or health supplement according to claim 16 wherein the complexes of phosphate derivatives of a micronutrient is added at a concentration of from 10 ppm to 10,000 ppm % (w/w).

21. A dietary or health supplement according to claim 20 wherein the micronutrient is added at a concentration of from 50 ppm to 1,000 ppm % (w/w).

22. A dietary or health supplement according to claim 16 selected from the group consisting of capsule, tablet, powder, food additives, cookie, biscuit, breakfast cereal, sports drink and sports food bar.

23. A dietary or health supplement according to claim 22 being a capsule.

24. A dietary or health supplement according to claim 23 containing tocopheryl phosphate arginine complex.

25. A dietary or health supplement according to claim 22 being a sports drink.

26. A dietary or health supplement according to claim 25 containing tocopheryl phosphate arginine complex.

27. A dietary or health supplement according to claim 22 being a cookie containing ubiquinyl phosphate.

28. A dietary or health supplement according to claim 27 containing tocopheryl phosphate arginine complex.

29. A method for supplementing a subject's intake of a daily allowance of a micronutrient selected from the group consisting of tocopherol, ubiquinol, ascorbic acid, tocotrienol, retinol and mixtures thereof, said method comprising administering to said subject a dietary or health supplement comprising an effective amount of the micronutrient in the form of one or more complexes of phosphate derivatives of the micronutrient delivered with an acceptable carrier.

30. Use of an effective amount of a micronutrient together with an acceptable carrier in the manufacture of a dietary or health supplement for supplementing a subject's intake of a daily allowance of the micronutrient, wherein the micronutrient is selected from the group consisting of complexes of phosphate derivatives of ubiquinol, ascorbic acid, retinol, tocotrienol, tocopherol and mixtures thereof.

31. A dietary or health supplement when used for supplementing a subject's intake of a micronutrient, the dietary or health supplement comprising an effective amount of one or more complexes of phosphate derivatives of ubiquinol, ascorbic acid, retinol, tocotrienol, tocopherol and mixtures thereof and an acceptable carrier.

32. A method for supplementing a subject's intake of a daily allowance of CoQ_{10}, said method comprising administering to said subject a dietary or health supplement comprising an effective amount of a mixture of ubiquinyl phosphate and di-ubiquinyl phosphate delivered with an acceptable carrier.

33. A method for supplementing a subject's intake of a daily allowance of vitamin E, said method comprising administering to said subject a dietary or health supplement comprising an effective amount of tocopheryl phosphate arginine complexes delivered with an acceptable carrier.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/01081

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A61K 31/6615, 31/661, A61P 31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See electronic database below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Medline, WPAT: ubiquin+, ascorb+, tocotrien+, retin+, tocopher+, +nutrient+, A61K, A23, vitamin E, vitamin A, phosphate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Fracalossi, DM, et al,"Oscars, Astronotus ocellatus, have a dietary requirement for vitamin C", J. of Nutrition, (Oct 1998), 128 (10), 1745-51. See especially pages 1745, 1746.	1, 3-7, 9, 13-16, 20-23, 25, 29-31
X	Blom J.H. et al, "Reproductive success of female rainbow trout (Oncorhynchus mykiss) in response to graded dietary ascorbyl monophosphate levels", Biology of Reproduction, (May 1995) 52 (5) 1073-80. See especially pages 1073-5	1, 3-7, 9, 13-16, 20-23, 25, 29-31

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
20 November 2002

Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/01081

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 565007 B (Senju Pharmaceutical Co. Inc.) 13 October 1993 Pages 3 and 4	1, 3-7, 9, 13-17, 19-25, 29-33
X	EP 684043 A (Senju Pharmaceutical Co. Inc.) 29 November 1995 Pages 2-6	1, 3-7, 9, 13-17, 19-25, 29-33
X	EP 845216 A (Showa Denko Kabushiki Kaisha) 3 June 1998 Pages 3-13	1, 3-7, 9, 13-17, 19-25, 29-33
X	US 5114957 A (Hendler, S. et al) 19 May 1992 Columns 2, claims 8 & 19	1, 3-7, 9, 13-17, 19-25, 29-33
X	US 5603949 A (Meybeck, A. et al) 18 February 1997 Columns 2-10	1, 3-7, 9, 13-17, 19-25, 29-33
X	US 5643597 A (Meybeck, A. et al) 1 July 1997 Columns 3-10	1, 3-7, 9, 13-17, 19-25, 29-33
X	WO 93/15731 A (Lamb, R.) 19 August 1993 Pages 3-12	1, 3-7, 9, 13-17, 19-25, 29-33
P, X	WO 02/26238 A (Tocovite Pt. Ltd.) 4 April 2002 Pages 2-17	1-17, 19-25, 27, 29-33

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU02/01081

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
EP	565007	CA	2091802	JP	5286848	US	5380753
EP	684043	CA	2146885	JP	7291870		
EP	845216	JP	10155429	US	6022867		
US	5114957	NONE					
US	5603949	EP	597025	FR	2679904	US	5643597
		WO	9302661				
US	5643597	EP	597025	FR	2679904	US	5603949
		WO	9302661				
WO	9315731	AU	36620/93				
WO	200226238	AU	20000393	AU	200193488	AU	20016847

END OF ANNEX